

REMARKS

Claims 1-5, 20, 23 and 26 are pending in the application. Claims 1-5 are withdrawn as being directed to non-elected inventions. Claims 20 and 23 are amended herein for clarity and to more particularly define the invention. In addition, withdrawn claims 1-5 are amended herein in order to put them into condition for rejoinder upon allowance of the product claims. Support for these amendments is found in the language of the original claims and throughout the specification, as set forth below. It is believed that no new matter is added by these amendments and their entry and consideration are respectfully requested. In light of these amendments and the following remarks, applicants respectfully request reconsideration of this application and allowance of the pending claims to issue.

I. Rejections under 35 U.S.C. § 112, second paragraph

The Office Action states that claims 20, 23, and 26 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Specifically, the Office Action states that the recitation in claims 20 and 23 of "a strand opposite the exogenous miRNA sequence" is confusing since it is unclear whether the recitation is referring to the nucleotides opposite the exogenous miRNA or to some other interpretation such as the entire strand opposite to the exogenous miRNA sequence is also replaced. The Office Action suggests replacing the term "a strand" with "nucleotides."

In addition, the Office Action states that claims 20 and 23 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for the recitations of "the secondary structure of the plant miRNA precursor" and "including double strandedness and mismatches in the same claim."

Claims 20 and 23 are amended herein to recite wherein the isolated plant miRNA precursor has been modified by (a) replacing the endogenous miRNA sequence of the isolated plant miRNA precursor with an exogenous miRNA sequence that maintains the length of the endogenous miRNA sequence; and (b) modifying nucleotides opposite the exogenous miRNA sequence in the isolated plant miRNA precursor to maintain double strandedness and mismatches of the plant miRNA precursor. Support for these

amendments can be found throughout the specification, for example, at least on page 5, lines 11-23; on page 6, lines 1-15; on page 9, lines 7-15 and 23-26; on page 10, lines 1-7; and on page 32, lines 11-20. Thus, no new matter is added by these amendments.

Accordingly, as suggested in the Office Action, claims 20 and 23 now recite "nucleotides" opposite the exogenous miRNA sequence instead of "a strand" opposite the exogenous miRNA sequence. Further, claims 20 and 23 no longer recite "secondary structure." Thus, applicants respectfully submit that claims 20 and 23 are now clarified and respectfully request that the rejection of these claims and claims dependent therefrom under 35 U.S.C. §112, second paragraph, be withdrawn.

II. Rejection under 35 U.S.C. § 103(a).

The Office Action states that claims 20, 23, and 26 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Cullen et al. (U.S. Patent Publication No. 2004/0053411) in view of Llave et al. (*Plant Cell* 14:1605-1619 (2002)) and Rheinhardt et al. (*Genes & Development* 16:1616-1626 (2002)). Specifically, the Office Action states that Cullen et al. teaches designing an artificial miRNA precursor by modifying a naturally occurring miRNA precursor sequence with an exogenous miRNA to a target. The Office Action further states that Cullen et al. teaches that said modification comprises incorporating an miRNA sequence of interest into said miRNA precursor by substituting stem sequences of its native miRNA to generate miRNAs suitable for use in inhibiting expression of any target gene of interest. Applicants respectfully disagree with the interpretation of Cullen et al.

As discussed previously, Cullen et al. has a filing date that is later than the priority date of the present application. However, as indicated in the Office Action, a provisional application was filed in the case of Cullen et al. (U.S. Provisional Application No. 60/377,224; hereinafter "the Cullen provisional") giving it an earlier priority date. Applicants note, however, that the Cullen provisional is not a complete disclosure of everything that is in Cullen et al. For example, while Cullen et al. mentions that plant miRNA precursors exist, the Cullen provisional fails to disclose any plant miRNAs or miRNA precursors and simply suggests that the invention "provides RNA molecules (miRNAs) functionally equivalent to siRNAs that can be transcribed endogenously in

animal and plant cells." (Cullen provisional, page 2, third full paragraph). However, no examples of plant miRNA precursors are provided in the Cullen provisional, only animal miRNA precursors are discussed. Thus, one of ordinary skill in the art would reasonably interpret this to mean that the constructs described in the Cullen provisional based on animal miRNA precursors are intended to function in a plant cell.

As the Office Action mailed October 31, 2006 in the case of the present application acknowledges, "differences exist in the structure and production of miRNA precursors from diverse sources, such as, animals, plants, metazoan etc. The differences include cellular compartmentalization, timing of precursor processing or types of cofactors involved in the processing of miRNA." Thus, miRNA precursors designed using animal miRNA precursors would not be expected to work in plant cells. Accordingly, one of skill in the art would recognize that the Cullen provisional does not teach the use of plant miRNAs and plant miRNA precursors.

In response, the Office Action argues that the Cullen provisional "suggest making virus resistant plants using modified miRNA molecules specifically designed to inhibit expression of plant viral genes." Page 5, Final Office Action of September 4, 2008. However, as stated above, the Cullen provisional fails to disclose any plant miRNAs or miRNA precursors and simply suggests that the invention "provides RNA molecules (miRNAs) functionally equivalent to siRNAs that can be transcribed endogenously in animal and plant cells." (Cullen provisional, page 2, third full paragraph). However, **no examples of plant miRNA precursors are provided in the Cullen provisional**, only animal miRNA precursors are discussed. Thus, one of ordinary skill in the art would reasonably interpret this to mean that the constructs described in the Cullen provisional based on animal miRNA precursors are intended to function in a plant cell.

Further, contrary to the contention in the present Office Action, Cullen et al. does not teach the present invention of a plant stably transformed with an miRNA precursor construct, said miRNA precursor construct comprising a promoter functional in a plant cell, wherein the promoter is operably linked to a nucleotide sequence encoding an isolated plant miRNA precursor, wherein the isolated plant miRNA precursor has been modified by (a) replacing an endogenous miRNA sequence of the isolated plant miRNA precursor with an exogenous miRNA sequence that maintains the length of the

endogenous miRNA sequence and (b) modifying nucleotides opposite the exogenous miRNA sequence in the isolated plant miRNA precursor to maintain double strandedness and mismatches of the plant miRNA precursor, and further wherein the exogenous miRNA sequence is complementary to a target mRNA sequence within said plant and, following processing from said plant miRNA precursor, hybridizes with the target mRNA sequence, whereby the expression of the target sequence is reduced.

Cullen et al. describes entirely artificial miRNAs and miRNA precursors. Cullen et al. does not describe an exogenous miRNA sequence that replaces an endogenous miRNA sequence, wherein the exogenous miRNA sequence maintains the length of the endogenous miRNA sequence as taught by the present invention. Further, Cullen et al. fails to teach or suggest that the nucleotides opposite the exogenous miRNA sequence in the isolated plant miRNA precursor are modified to maintain the double strandedness and mismatches of the plant miRNA precursor, as is now claimed in the present invention. Cullen et al. does not teach or suggest that the secondary structure of the native miRNA precursor is to be considered at all in the designing of the artificial miRNA precursor constructs described therein and the examples provided in Cullen et al. clearly illustrate that this is the case.

For instance, Figure 3A of Cullen et al. illustrates the designed miRNA of Cullen et al. based on the miR-30 precursor and in this case the length and the central bulge found in the native miR-30 is not maintained in the artificial miRNA (native miR-30 precursor is shown in Figure 1 A of Cullen et al.). Figure 5 provides additional examples where Cullen et al. has designed artificial miRNA precursors without regard to maintenance of the precursor secondary structure or miRNA length as compared to the native miRNA precursor, miR-30. In many of the examples in Figure 5, no bulge is present at all, and even when the bulge is present, it is not in the same position nor is it the same size as that found in the native miR-30 precursor.

Nowhere in the text of Cullen et al. is it taught or suggested that maintenance of the double strandedness and mismatches is of concern and, in fact, the contrary is taught. Indeed, no importance is attached to maintenance of the overall structure of the miRNA precursor as is evident from the following passage:

...while the presence of a miR-30 loop may be desirable, variations of that structure can also be tolerated (e.g., loops can be used that are greater than 72%, preferably greater than 79%, more preferably greater than 86%, and most preferably, greater than 93% identical to, for instance, the miR-30 sequence...).

Cullen et al., paragraph 0023.

Accordingly, Cullen et al. teaches a wide variation in the designing of an miRNA precursor. Such variation as suggested by Cullen et al. would be recognized by one of ordinary skill in the art to have the effect of destroying not only the double strandedness and mismatches but most of the overall structure of the native miRNA precursor.

In response, the Final Office Action asserts that "it would have been obvious and within the scope of an ordinary skill in the art to maintain the secondary structure of the modified plant miRNA precursor because it was unknown what the effect of removing the secondary structure would be, it would have been obvious not to alter it." Page 7, Final Office Action of September 4, 2008. Besides ignoring the fact that nowhere in the text of Cullen et al. is it taught or suggested that maintenance of the double strandedness and mismatches is of concern, the above statement of the Office Action dismisses the fact that Cullen et al. explicitly states that "variations in the structure can be tolerated." Para. [0023]. As such, it is respectfully submitted that the presently pending claims patentably define over Cullen et al.

The Office Action additionally states that Cullen et al. teaches that the modification of the naturally occurring miRNA precursor "comprises incorporating an miRNA sequence of interest into said miRNA precursor by substituting stem sequences of its native miRNA to generate miRNAs suitable for use in inhibiting expression of any target gene of interest...." (Action, page 11, emphasis added). However, Cullen et al., in fact, states "the stem sequence in the miR-30 precursor was substituted with a sequence based on the *Drosophila* *next* gene." (Cullen et al., paragraph 52). Thus, Cullen et al. teaches substituting the entire miR-30 stem sequence, not just the miRNA sequence found within the stem, as alleged in the Office Action. Nowhere in Cullen et al. is it stated that only the endogenous miRNA sequence within the stem sequence is replaced by an exogenous miRNA sequence with nucleotides opposite the exogenous

miRNA sequence in the isolated plant miRNA precursor being modified to maintain the double strandedness and mismatches of the plant miRNA precursor as is claimed in the present invention.

Further, the resultant miRNA precursor described in paragraph 52 of Cullen et al. is shown in Figure 3A. As discussed previously, this artificial miRNA clearly does not show a native miRNA sequence wherein the endogenous miRNA sequence within the stem is replaced with an exogenous miRNA sequence while maintaining the length of the endogenous miRNA and the double strandedness and mismatches of the native miRNA precursor as claimed in the present invention. Figure 3A of Cullen et al. shows a designed miRNA that is missing the bulge of the endogenous miRNA and having a length that is different from the endogenous miRNA sequence.

In further support of the contention that Cullen et al. teaches substitution of the entire stem, applicants direct the Examiner's attention to a citation from the Cullen provisional, wherein it states that "[a]dvantageously, the entire miRNA containing stem is complementary to the target sequence, even in regions of the stem 3' and 5' to the miRNA." (Cullen provisional, page 7, lines 7-9). Thus, it is obvious that the Cullen provisional teaches that the entire miRNA-containing stem be replaced, not just the endogenous miRNA sequence, as is taught by the present invention. Clearly, the Cullen provisional upon which Cullen et al. derives its priority date as well as Cullen et al. itself teach away from a miRNA precursor wherein the endogenous miRNA sequence within the stem is replaced with an exogenous miRNA sequence while maintaining the length of the endogenous miRNA and the double strandedness and mismatches of the native miRNA precursor as claimed in the present invention.

It is unclear why the Office Action fails to note that Cullen et al. does not take into consideration and, in fact, teaches away from, maintaining the double strandedness and mismatches of the miRNA precursors. Applicants find this particularly surprising since throughout the prosecution of the present application, the Examiner has repeatedly raised the issue of secondary structure in the designing of artificial miRNAs. For example, in the Office Action mailed October 31, 2006 in the present application, it is stated that "[d]esigning a miRNA precursor comprising a sequence which is complementary to the target sequence of interest would require prediction analysis of

said precursors to form an irregular hairpin structure containing various mismatches, internal loops and bulges so that the predicted precursor molecule forms most favorable structure in solution. This involves extensive analysis of secondary structures of the predicted miRNA precursors and selecting the structure with the lowest free energy so that predicted non-natural miRNA precursor is effectively processed...." Cullen et al. mentions the possibility of including bulges but never within the context of the endogenous miRNA precursor secondary structure and as discussed above, in most examples shown in Cullen et al. the artificial miRNA precursors violate one or more aspects of the secondary structure of the native miRNA precursor (see, e.g., Figures 1A, 3A and 5A of Cullen et al.).

Finally, applicants note that the only mention in Cullen et al. of maintaining any of the structure of a naturally occurring miRNA precursor sequence is found in paragraph 0023. However, not only is this paragraph not prior art to the present invention because it is not found in the Cullen provisional but this paragraph still fails to teach or suggest the present invention as it suggests the entire stem sequence be replaced not just the miRNA (similar to Cullen et al. paragraph 52, discussed above)) and then goes on to suggest that variations in loop structure can be tolerated. Cullen et al. simply does not teach or suggest designing miRNA precursors with the features of an endogenous miRNA precursor but with the endogenous miRNA sequence being replaced with an exogenous miRNA sequence wherein the exogenous miRNA sequence maintains the length of the endogenous miRNA sequence and nucleotides opposite the exogenous miRNA sequence in the isolated plant miRNA precursor are modified to maintain the double strandedness and mismatches of the plant miRNA precursor as claimed in the present invention.

Further, Llave et al. and Rheinhardt et al. fail to remedy the deficiencies of Cullen et al. Llave et al. and Rheinhardt et al. discuss native plant miRNA precursors. Neither reference teaches or suggests a plant stably transformed with an miRNA precursor construct, said miRNA precursor construct comprising a promoter functional in a plant cell, wherein the promoter is operably linked to a nucleotide sequence encoding an isolated plant miRNA precursor, wherein the isolated plant miRNA precursor has been modified by (a) replacing an endogenous miRNA sequence of the isolated plant miRNA

precursor with an exogenous miRNA sequence that maintains the length of the endogenous miRNA sequence and (b) modifying nucleotides opposite the exogenous miRNA sequence in the isolated plant miRNA precursor to maintain double strandedness and mismatches of the plant miRNA precursor, and further wherein the exogenous miRNA sequence is complementary to a target mRNA sequence within said plant and, following processing from said plant miRNA precursor, hybridizes with the target mRNA sequence, whereby the expression of the target sequence is reduced as claimed in the present invention.

The Office Action alleges that based on the many native miRNA sequences that contain mismatches or bulges seen in Llave et al. and Rheinhardt et al., it would have been obvious to maintain the size and positions of mismatches of the native miRNA secondary structure in the non-native miRNA sequence of the modified plant miRNA precursor to avoid any possible problems during processing of the miRNA precursor. Final Office Action of September 4, 2008, page 8-9. However, the Office Action provides no basis upon which this conclusion could be made. Nowhere in any cited reference or in the general knowledge of those of ordinary skill in the art at the time this application was filed was the maintenance of the size of the miRNA and the positions of mismatches found in the native miRNA taught or suggested as a way to avoid processing problems or for any other reason. In fact, contrary to the contention of the Office Action, one of ordinary skill in the art at the time the present application was filed could easily have concluded just the opposite from the disclosures of Llave et al. and Rheinhardt et al., i.e., that it was unnecessary to maintain double strandedness and mismatches because the various different native miRNA precursors of Llave et al. and Rheinhardt et al. show variable miRNA sizes and positions of mismatches. Clearly, the skilled artisans in the case of Cullen et al. (filed only weeks prior to the filing of the present invention) made the conclusion that maintenance of secondary structure was unnecessary.

Accordingly, applicants respectfully submit that the present invention is non-obvious in view of Cullen et al., Llave et al. and/or Rheinhardt et al. as they fail to teach or suggest all of the recitations of the claims as required for a rejection under 35 U.S.C. § 103. Therefore, applicants respectfully request the withdrawal of this rejection.

III. Rejoinder of claims 1-5.

Claims 1-5 as presented herein include all of the recitations of product claims 20, 23 and 26. Thus, if it is determined that the products of claims 20, 23 and 26 are allowable, applicants request review and examination of these method claims in the present application, pursuant to the practice of rejoinder as set forth in section 821.04 of the MPEP. In particular, it is stated therein that if a product claim is elected in a restriction and then found allowable, withdrawn process claims that depend from or otherwise include all of the limitations of the allowable product claim are to be rejoined in the same application.

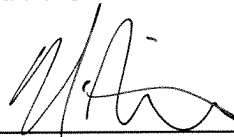
In summary, Applicants respectfully submit that the present claims patentably define over all of the prior art of record for at least the reasons set forth above. As such, it is believed that the present application is in complete condition for allowance and favorable action, therefore, is respectfully requested. Examiner Kumur is invited and encouraged to telephone the undersigned, however, should any issues remain after consideration of this Amendment.

Please charge any additional fees required by this Amendment to Deposit Account No. 04-1403.

March 4, 2009

Date

Respectfully submitted,
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